pendix "G"

PATENT Case 5400/2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF	
S. BERTENSHAW ET AL	GROUP ART UNIT: 120
SERIAL NO.: 08/425,022	EXAMINER: DENTZ
FILED: April 19, 1995	DATE: APRIL 15, 1997

TITLE: SUBSTITUTED FURANS AND FURANONES FOR THE TREATMENT OF INFLAMMATION

DECLARATION UNDER 37 C.F.R. §1.132

The Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

I, Gideon Fraenkel, Ph.D., declare that:

1. I received a Bachelor of Science Degree in Chemistry from the University of Illinois in 1952; received a Masters of Science in Chemistry from Harvard University in 1953, and received a Ph.D. in Chemistry from Harvard University, in 1957;

2. I was a Research Fellow with the California Institute of Technology from 1957-60.

3. Since 1960, I have been on the faculty at the Ohio State University of in Columbus, Ohio. Currently I hold the position of Professor of Chemistry, and direct eleven scientists carrying out research in nuclear magnetic resonance spectroscopy (NMR);

4. I am the principal author or co-author of approximately 150 publications, with several publications on NMR techniques, and specifically NMR determination of dynamic systems;

5. In my professional capacities, I closely and carefully follow the scientific literature regarding organic chemistry, and specifically nuclear magnetic spectroscopy (NMR) methods;

6. I have studied the NMR data acquired by Dr. John Likos on various substituted 4-(4-methylsulfonyl)phenyl-furans and attached hereto as Appendix "A" (pages A1-A12). Based on my study, I have found the following results:

a. On page A1, the observed chemical shifts are consistent with the keto tautomer of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone (A1(a)). Upon addition of base, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), two distinct sets of signals are observed (A1(b)). In addition to resonances associated with the keto tautomer, other observed proton resonances are consistent with the enol tautomer (δ =7.71 (d, 2H, J=8.38 Hz,) and 7.34 (d, 2H, J=8.38 Hz.) assigned to the 4-(4-methylsulfonyl)phenyl group, 7.12 (d, 2H, J=7.52 Hz.) assigned to H2 and H6 of the 3-phenyl group, 6.96 (t, 2H, J=7.52 Hz.) assigned to H3 and H5 of the 3-phenyl group, 6.66 (t, 1H, J=7.52 Hz.) assigned to H4 of the 3-phenyl group, 6.55 (s, 1H) assigned to H5 of the furan ring, and 3.12 (s, 3H) assigned to the methylsulfonyl protons);

b. On page A2, a ^{1}H-COSY (correlated spectroscopy) spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone is shown. The proton coupling patterns for the aromatic rings are indicated for the furanone (solid line) and the furanol (dashed line). The coupling patterns indicate that both keto and enol forms are present, consistent with the assignments made above;

c. On page A3, the DEPT-HMQC spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone and DBU indicates that the resonance at 6.55 ppm is a methine (CH) proton (A3(a)). This study confirms the assignment of the furanol H5 proton;

d. On page A4, a NOESY spectrum (NOE spectroscopy) of 4-(4-methylsulfonyl) phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O is shown. The cross-peaks between the methylsulfonyl group (3.16 ppm) and the resonance at 7.87 ppm indicates that this resonance

is H5 and H3 of the 4-(4-methylsulfonyl)phenyl group due to the proximity of the protons. Likewise, the resonance at 7.56 ppm is assigned to H2 and H6 of the 4-(4-methylsulfonyl)phenyl group due to their proximity to the furanone H5 protons. In addition, the cross-peak between H2 and H6 of the 4-(4-methylsulfonyl)phenyl (7.56 ppm) and the resonance at 7.28 ppm suggests that the 7.28 ppm resonance corresponds with H2 and H6 of the 3-phenyl group. These assignments are consistent with and extend the assignments presented above for the keto form;

e. On page A5, a NOESY spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone and DBU is shown. In addition to the negative NOE peaks seen for the keto form (discussed above), positive cross-peaks are observed. The presence of these cross-peaks due to chemical exchange indicates that the enol/keto tautomers are interconverting;

f. On page A6, the NMR spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone and sodium hydroxide is shown. Two distinct sets of resonances are observed that correlate with both furanone and furanol forms. Upon neutralizing this solution with HCl, the enol resonances decrease and two distinct sets of peaks are seen (6(b)) that correlate with two different furanone forms. In addition to the previously described conjugated ($\alpha\beta$) keto form, the unconjugated ($\beta\gamma$) keto form is observed with proton resonances at δ =8.08 (d, 1H, J = 2.1Hz.) and 5.27 (d, 1H, J = 2.1 Hz.) (assigned to the H3/H5 pair of protons), 7.74 (d, 2H, J = 8.76 Hz.) and 7.52 (d, 2H, J = 8.76 Hz.) (assigned to the 4-(4-methylsulfonyl)phenyl group), 7.32 (m) and 7.25 (m) (assigned to the 3-phenyl group), and 3.08 (s, 3H) (assigned to the methylsulfonyl group). The chemical shifts, J-couplings and integration of these new resonances are consistent with the unconjugated keto form;

g. On page A7, the DEPT-HMQC spectrum (A7(a)) of the neutralized mixture indicates that the resonances at 8.08

and 5.27 ppm are both methine protons (CH), consistent with their assignment in the unconjugated keto form;

h. On page A8, the NMR spectrum of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone is shown (A8(a)). The keto tautomer is observed with resonances at δ =8.23 (d, 2H, J = 8.89 Hz.), 7.57 (d, 2H, J = 8.89 Hz.), 7.89 (d, 2H, J = 8.53 Hz.), and 7.56 (d, 2H, J = 8.53 Hz.) (assigned to the aryl protons), 5.39 (s, 2H) (assigned to H5), and 3.16 (s, 3H) (assigned to the methylsulfonyl group). Upon addition of DBU (~0.8 equiv.), a second distinct set of signals are observed at δ =7.81 (d, 2H, J = 8.37 Hz.), 7.41 (d, 2H, J = 8.37 Hz.), 7.72 (d, 2H, J = 9.20 Hz.), and 7.26 (d, 2H, J = 9.20 Hz.) (assigned to the aryl protons), 6.68 (s, 1H) (assigned to H5 of the furanol ring), and 3.15 (s, 3H) (assigned to the methylsulfonyl group). The chemical shifts, J-couplings and integration are consistent with the enol tautomer;

i. On page A9, the DEPT-HMQC spectrum (A9(a)) of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone and DBU (~0.8 equiv.) in d_6-DMSO/H_2O (89.5/10.5 v/v%) indicates that the resonance at 6.68 ppm is a methine (CH) proton consistent with its assignment as H5 of the enol tautomer;

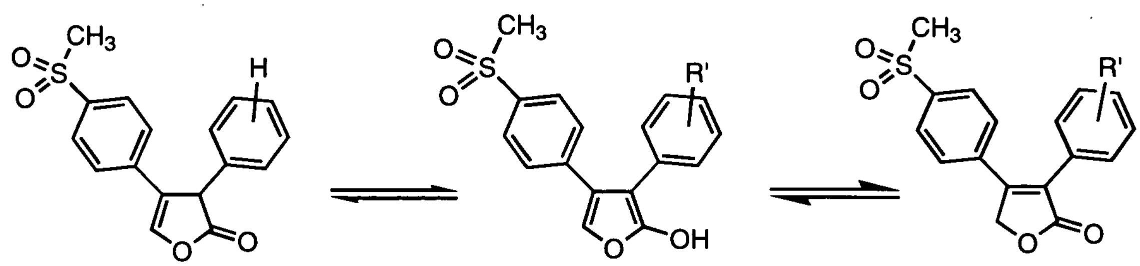
j. On page A10, the NMR spectrum of 3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone is shown (A10(a)). Signals attributed to the keto tautomer are observed at δ =7.88 (d, 2H, J = 8.70 Hz.) and 7.55 (d, 2H, J = 8.70Hz.) (assigned to the 4-(4-methylsulfonyl)phenyl group), 7.33 (q, 2H, J_{HH} = 8.84 Hz., J_{HF} = 5.52 Hz.) and 7.21 (t, 2H, J_{HH} = 8.84 Hz., J_{HF} = 8.84 Hz.) (assigned to the 3-(4-fluorophenyl) group), 5.32 (s, 2H) (assigned to H5), and 3.16 (s, 3H) (assigned to the methylsulfonyl group). Upon addition of DBU (~0.7 equiv.), a second distinct set of signals is observed at δ=7.71 (d, 2H, J = 8.41 Hz.) and 7.32 (d, J = 8.41 Hz.) (assigned to the 4-(4-methylsulfonyl)phenyl group), 7.09 (q, 2H, J_{HH} = 9.04 Hz., J_{HF} = 5.74 Hz.) and 6.78 (t, 2H, J_{HH} = 9.04 Hz., J_{HF} = 9.04 Hz.) (assigned to the 3-(4-fluorophenyl)group), 6.54 (s, 1H) (assigned to H5), and 3.11 (s, 3H) (assigned to the methylsulfonyl group).

The chemical shifts, J-couplings and integration are consistent with the enol tautomer;

k. On page A11, NMR data pertaining to the deuterium exchange reaction of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone are given. On page A11(a) the spectral changes observed during the course of the reaction are shown. The signal intensity of the keto tautomer H5 protons (5.40 ppm) decreases with time while a signal at 5.38 ppm initially increases then decreases. This 5.38 ppm resonance is assigned to the H5 mono-deuterated keto tautomer. A plot of the normalized signal intensities is shown in A11(b). These data are consistent with the formation of the enol tautomer during the exchange reaction.

l. On page A12, NMR data pertaining to the deuterium exchange reaction of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone are given. Figure A12(a) shows the changes observed during the course of the reaction. The signal intensity of the H5 protons (5.40 ppm) of both the keto and furanol tautomers decrease with time, while the signal assigned to the H5 mono-deuterated carbonyl tautomer (5.38 ppm) initially increases then decreases. A plot of the normalized signal intensities are shown in A12(b). These data show the compound incorporates deuterium at position C5 of the furanone, which is consistent with the formation of a 2-hydroxy compound during the exchange reaction;

7. These NMR results clearly show the presence of the three tautomeric structures depicted below (where R′ is fluoro, hydrogen or nitro):



keto enol keto

I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

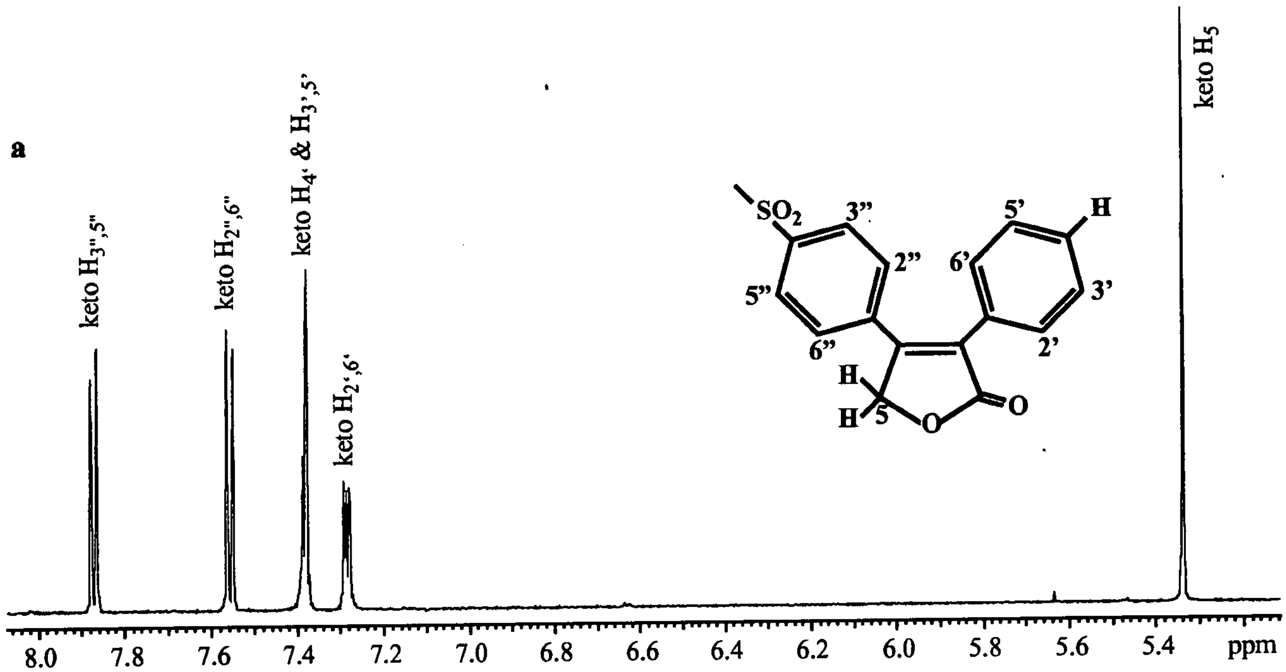
Respectfully submitted

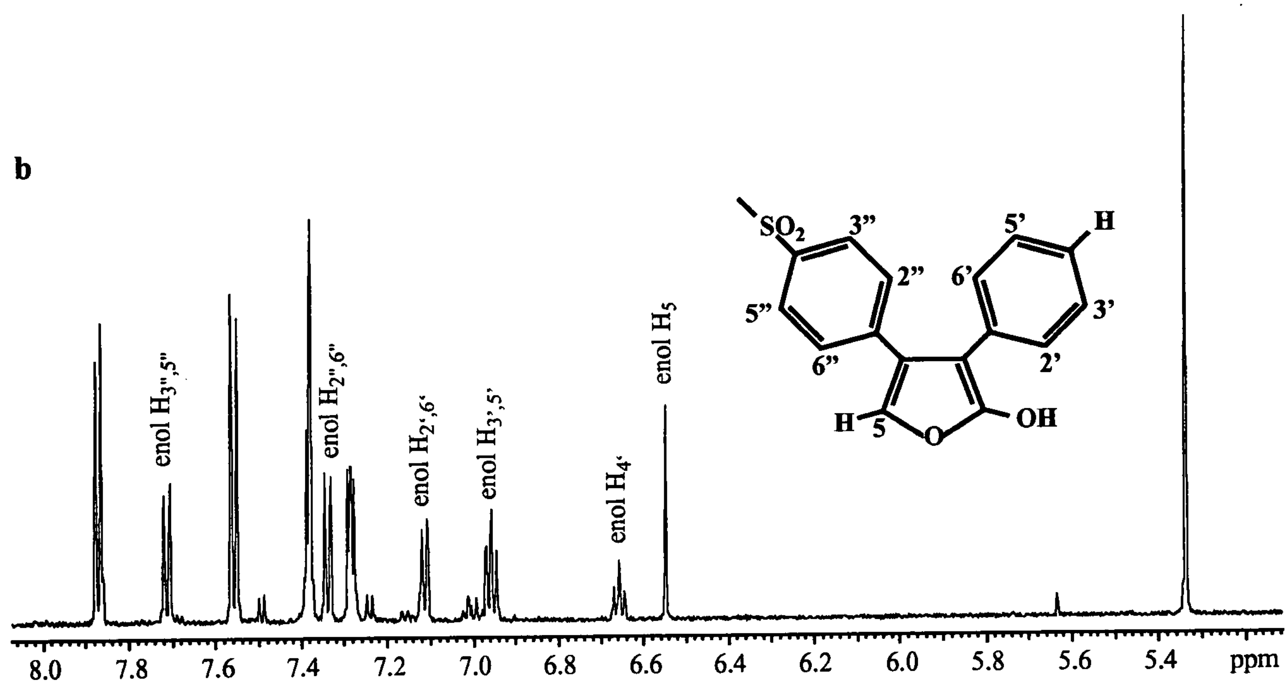
4-18-97

Date

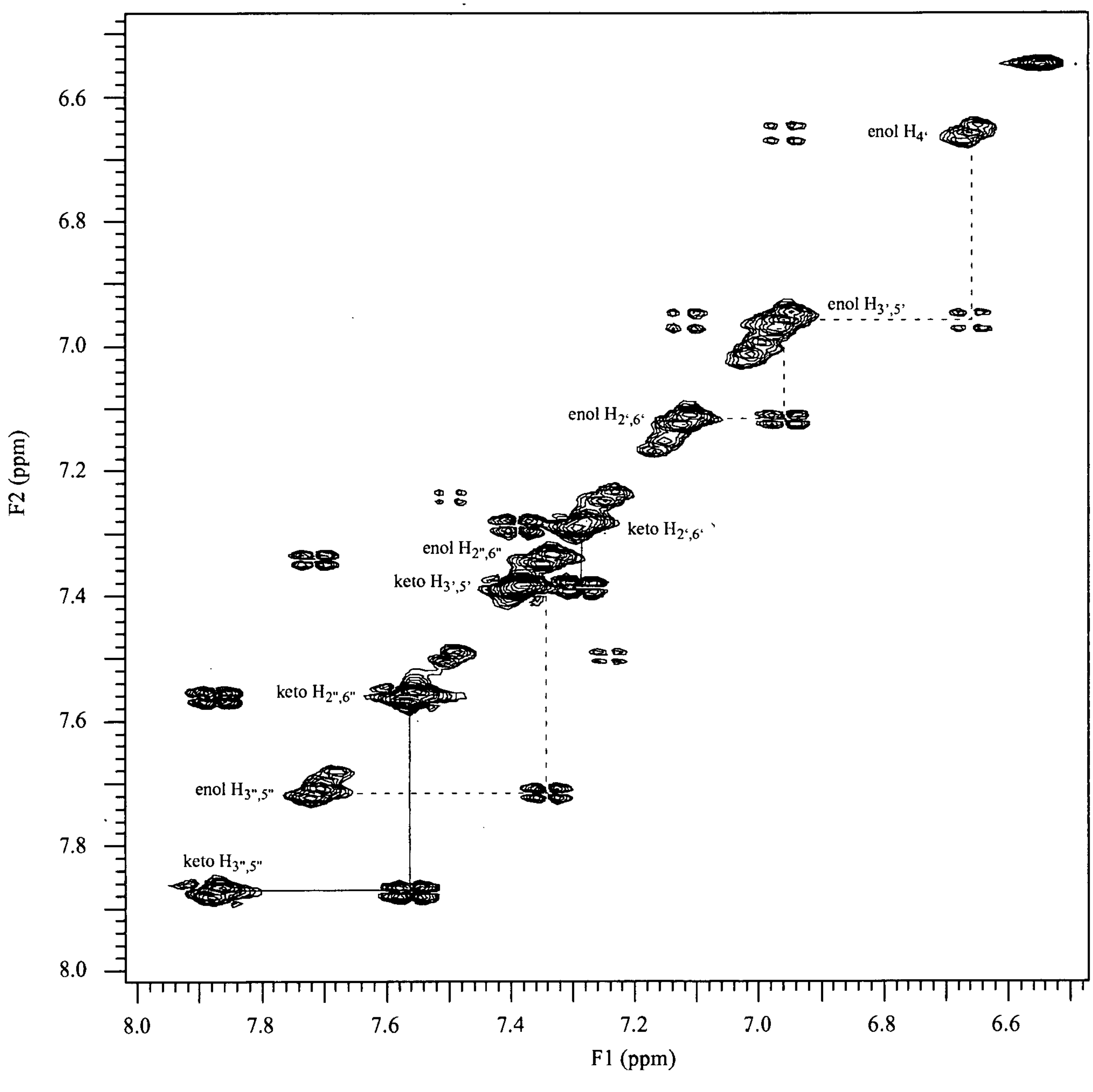
Gideon Fraenkel

Gideon Fraenkel, Ph.D.

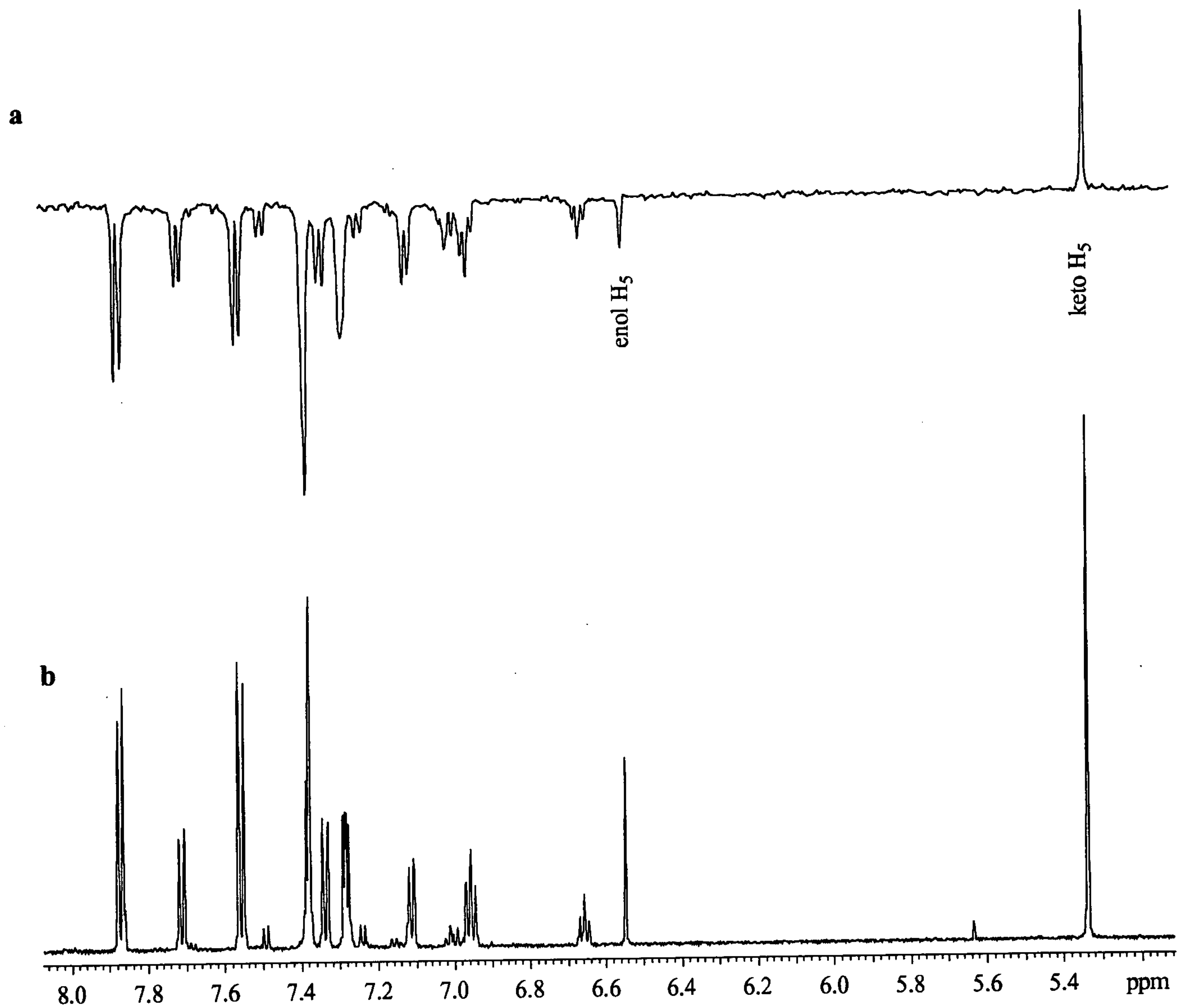




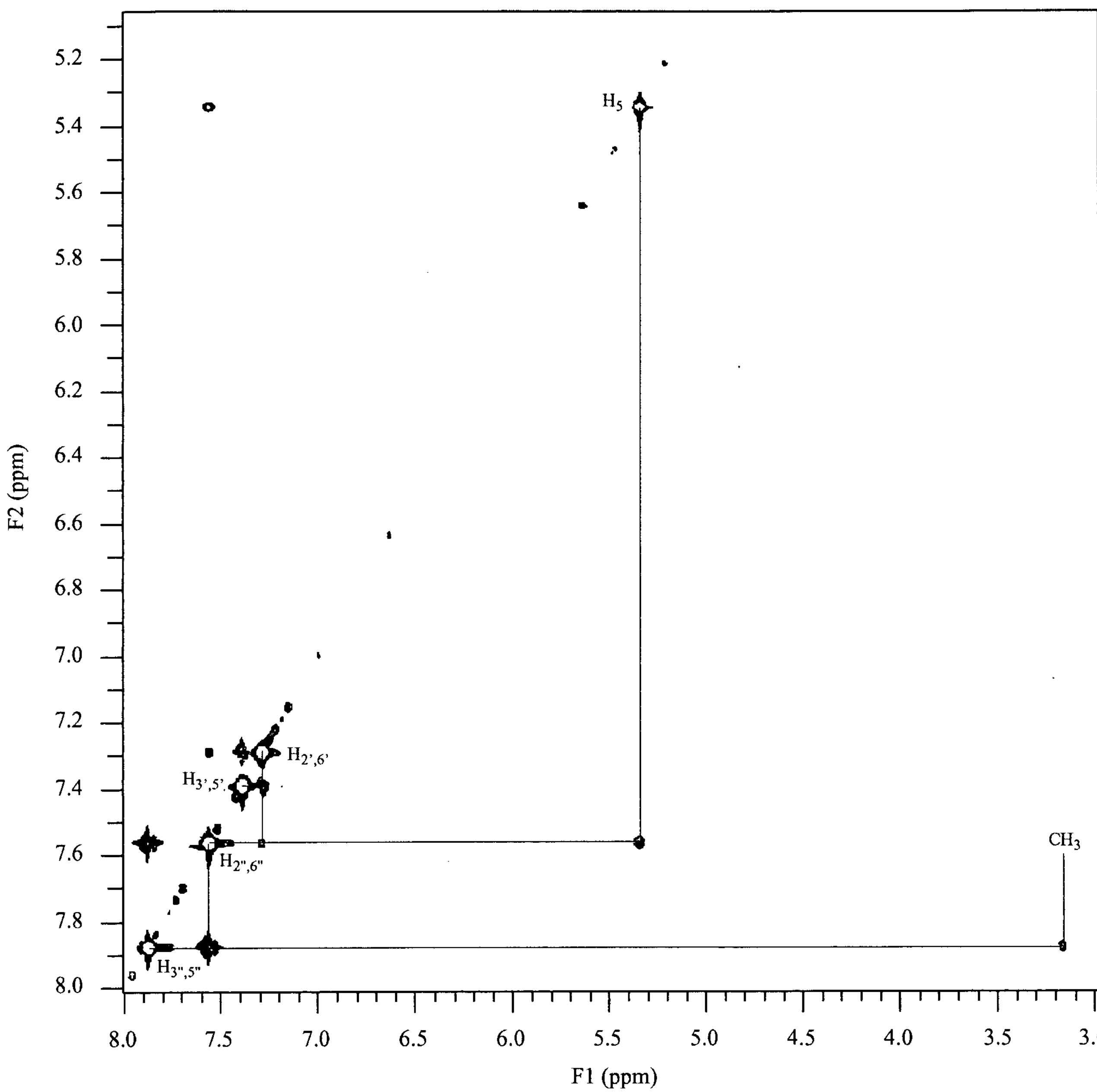
a. The ^{1}H NMR spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%);
b. after addition of DBU (~1.4equiv.) to (a).



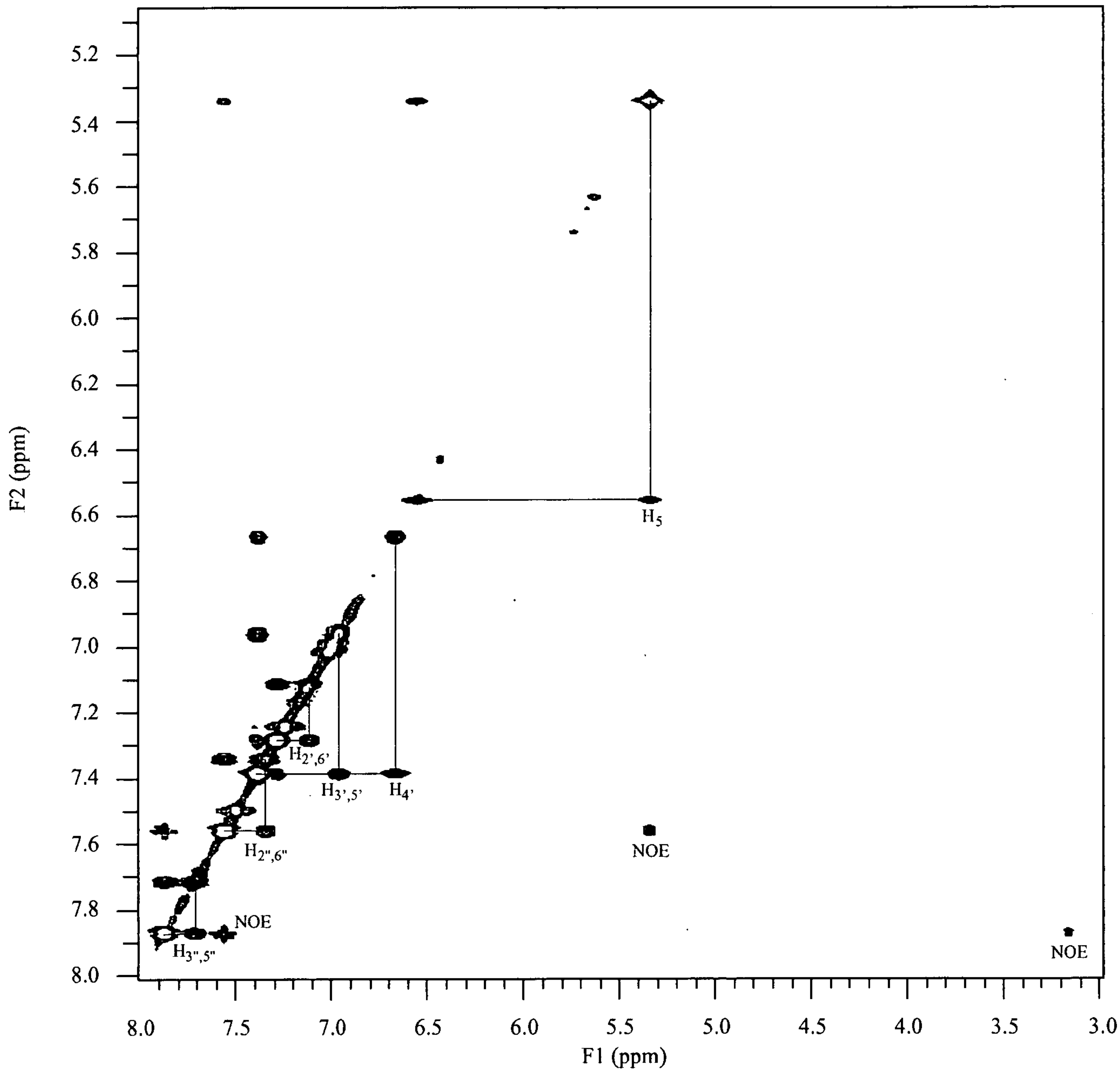
The ^{1}H-COSY spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%) after addition of DBU (~1.4equiv.). The solid lines indicate the aromatic ^{1}H couplings in the keto form; the dashed lines, in the enol.



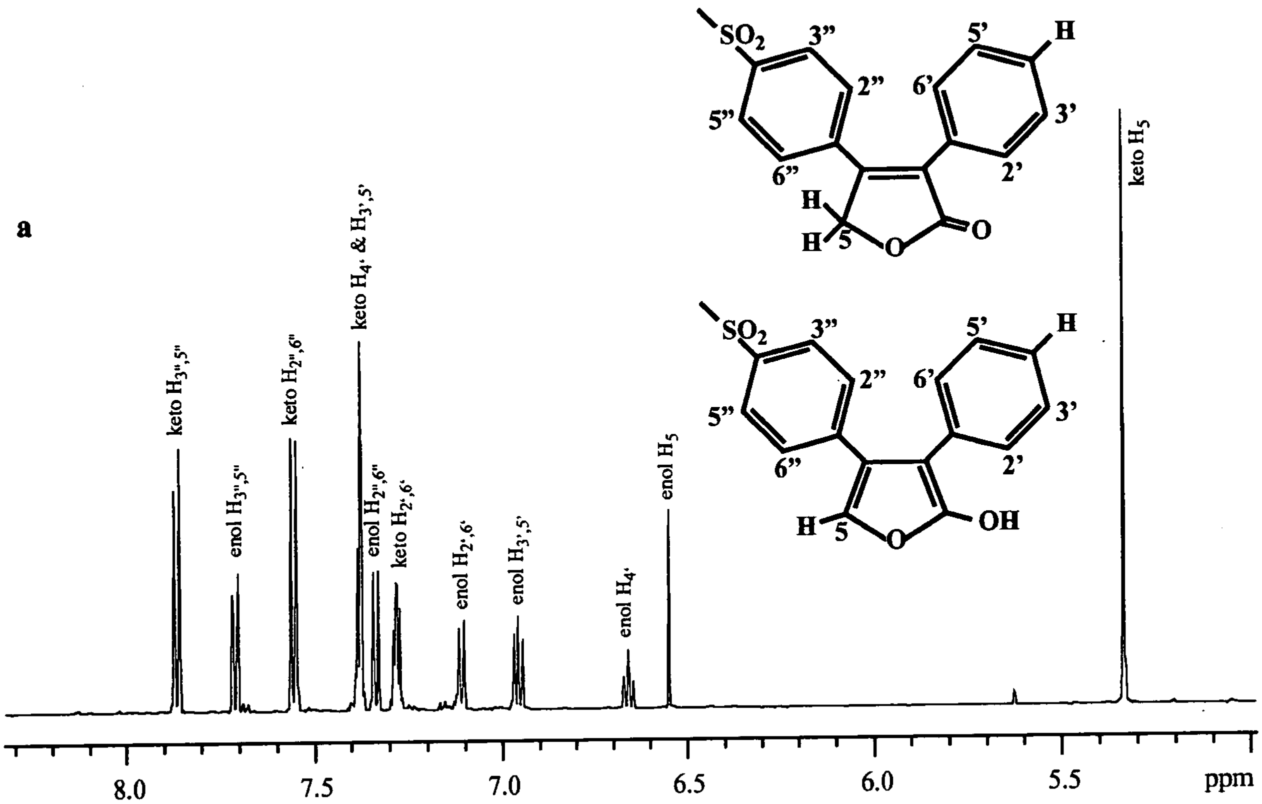
a. The [1]H-DEPT-HMQC spectrum of mixture from A1(b); CH_2 groups are up, CH and CH_3 down;
b. spectrum of A1(b) shown for reference.

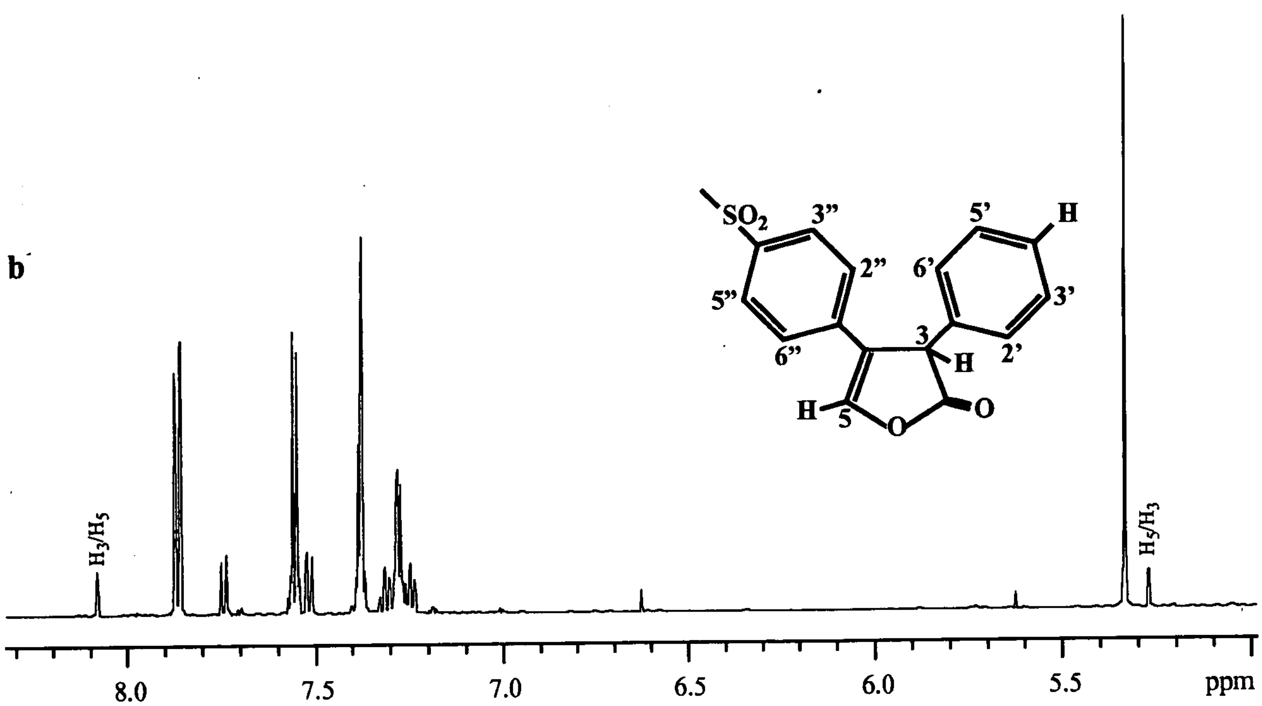


The ^{1}H-NOESY spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%).

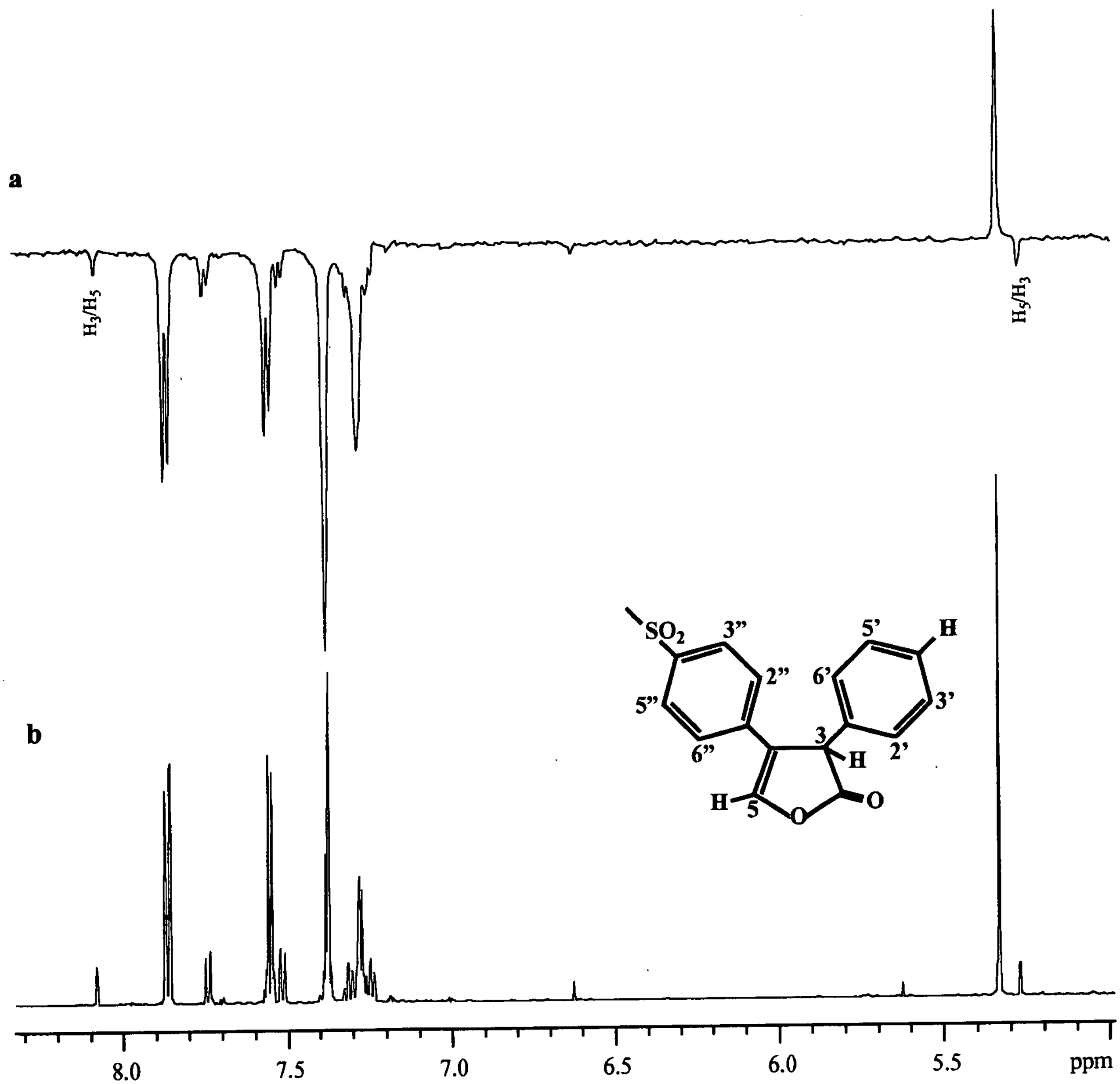


The ^{1}H-NOESY spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%) after addition of DBU (~0.7 equiv.). The lines connect pairs of keto/enol protons whose longitudinal magnetization is mixed by two-site chemical exchange.

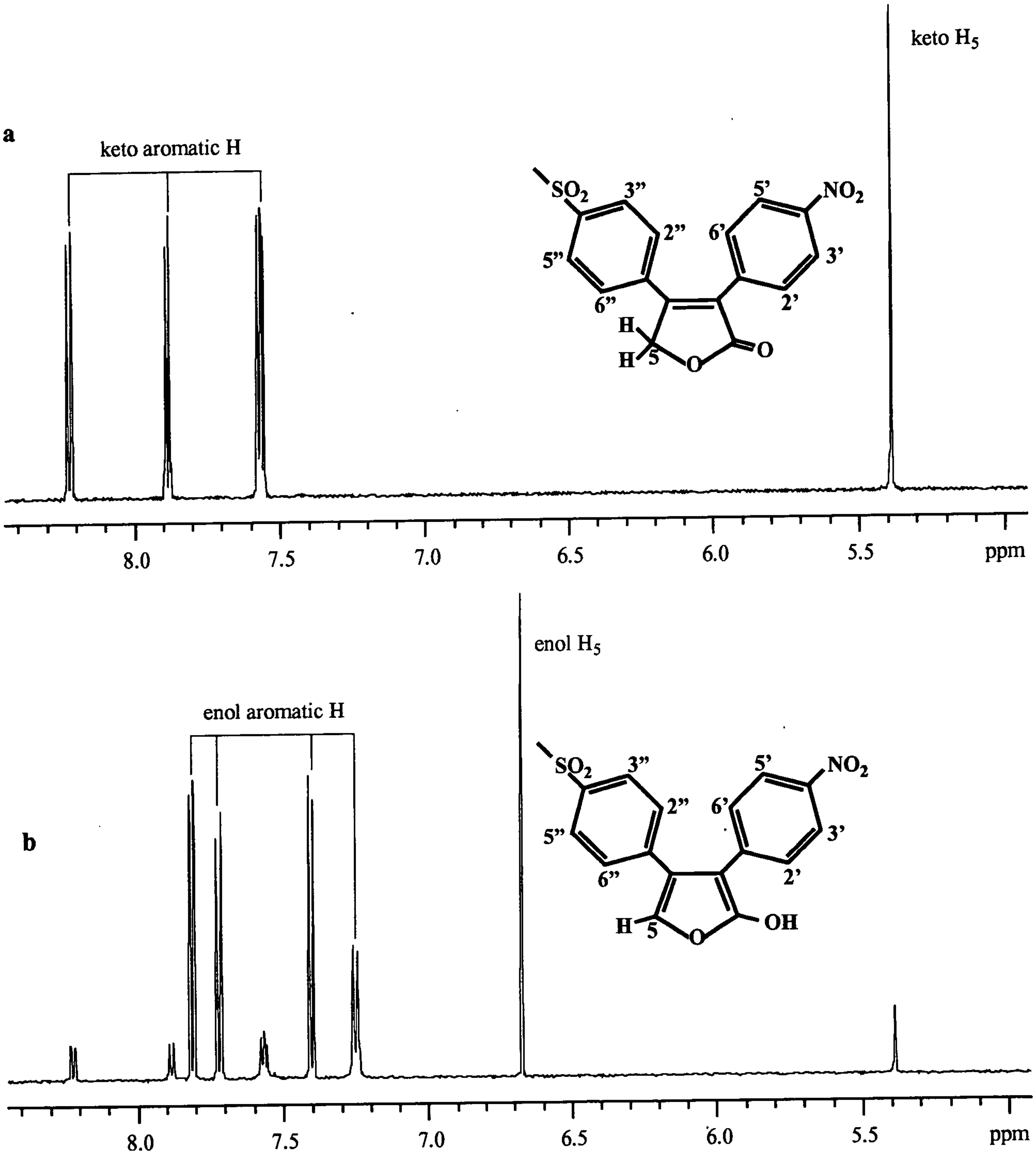




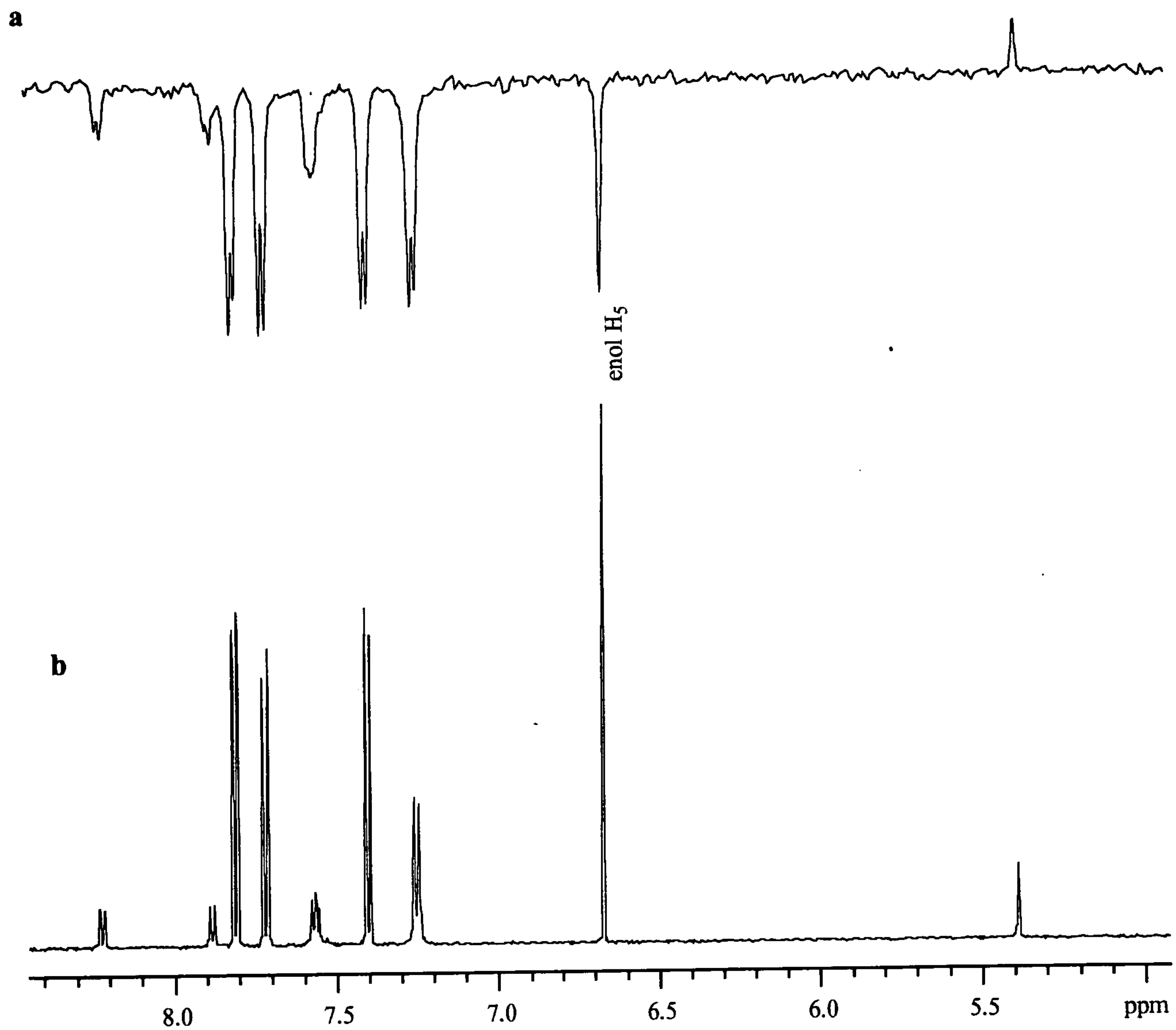
a. The ^{1}H-NMR spectrum of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%) after addition of NaOH (~0.5 equiv.);
b. after neutralizing (a) with HCl.



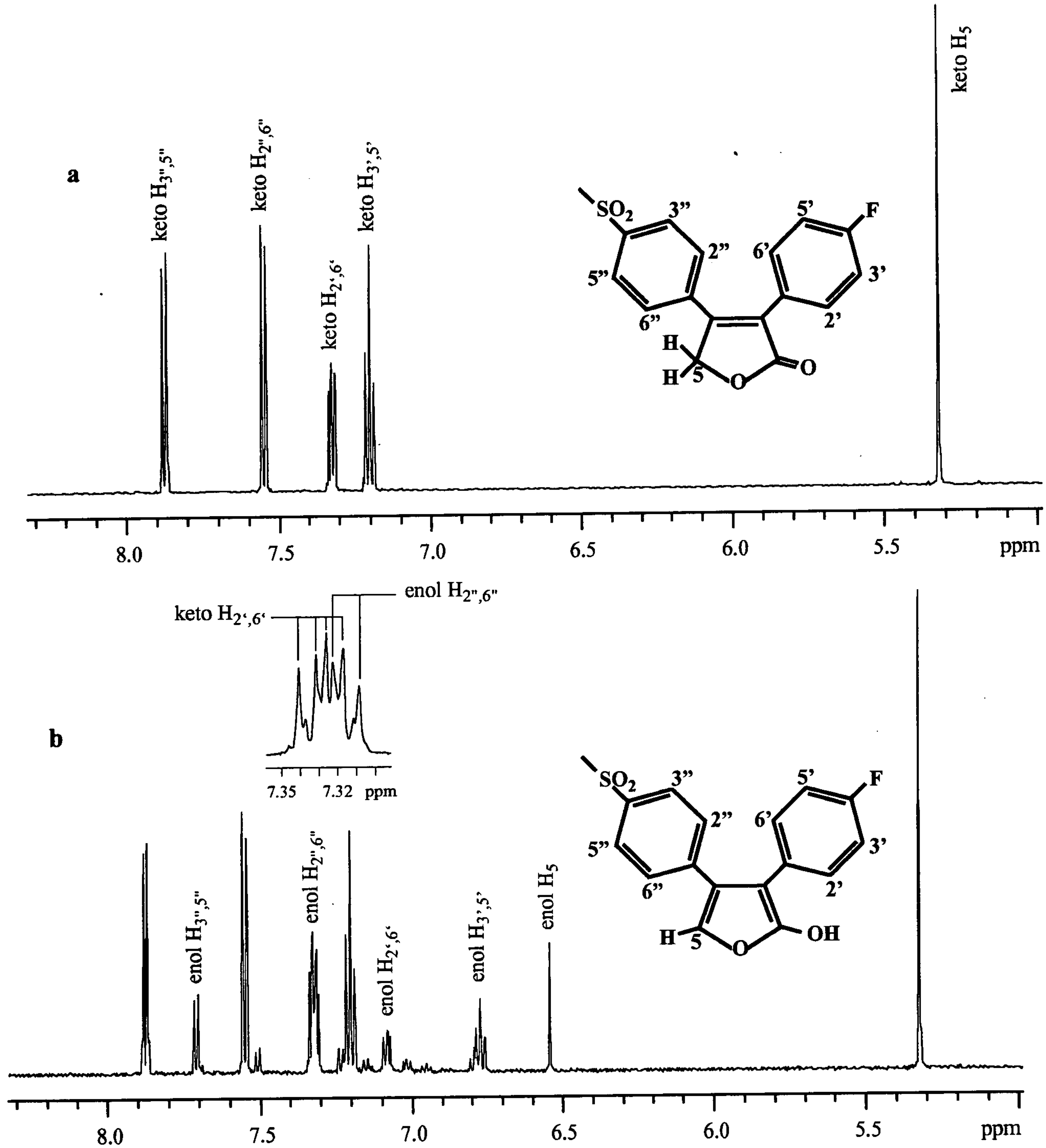
a. The ^{1}H-DEPT-HMQC spectrum of the mixture from A6(b); CH_2 groups are up, CH and CH_3 down; **b.** spectrum of A6(b) shown for reference.



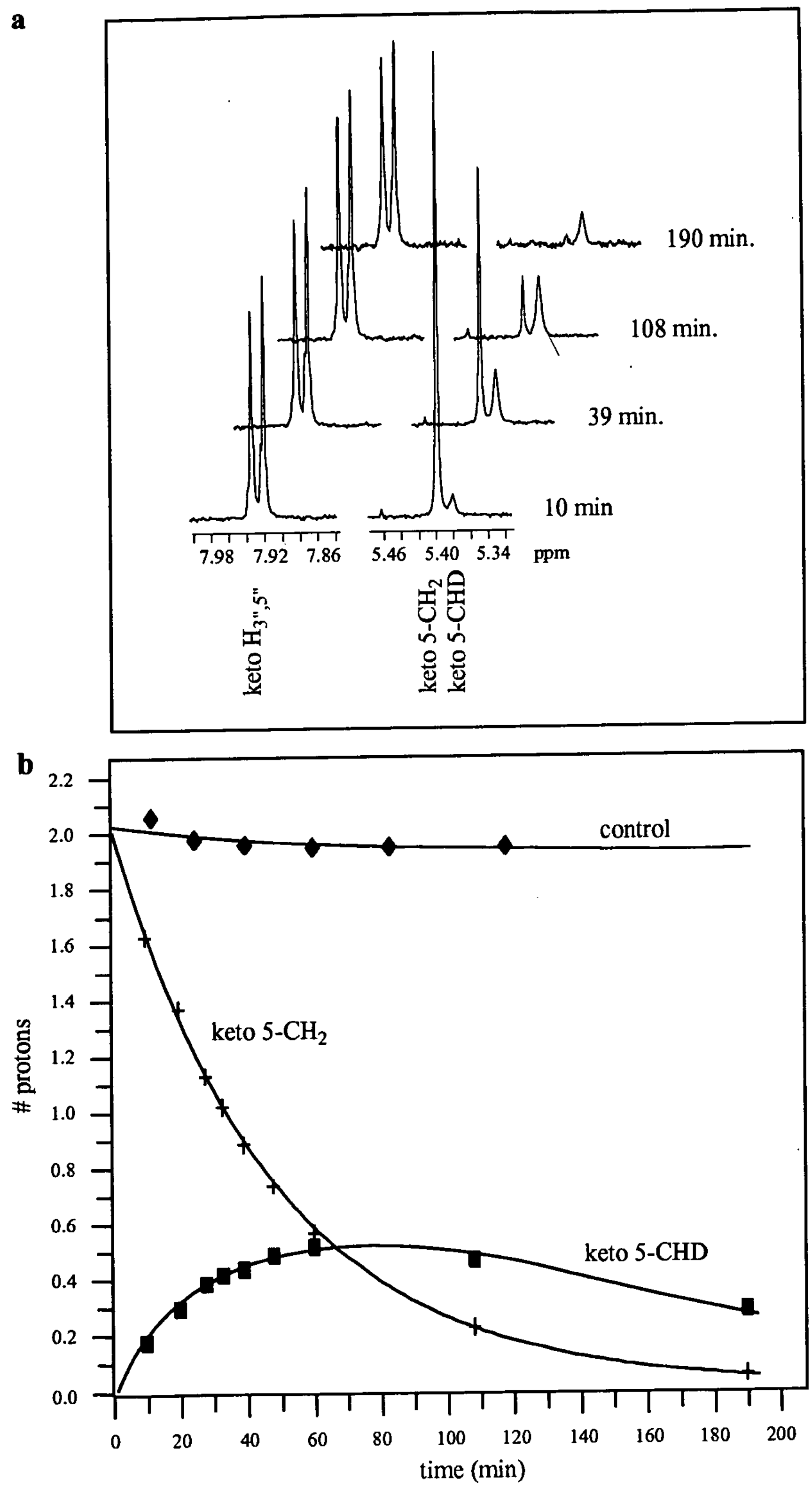
a. The ^1H NMR spectrum of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%);
b. after addition of DBU (~ 0.8 equiv.) to (a).



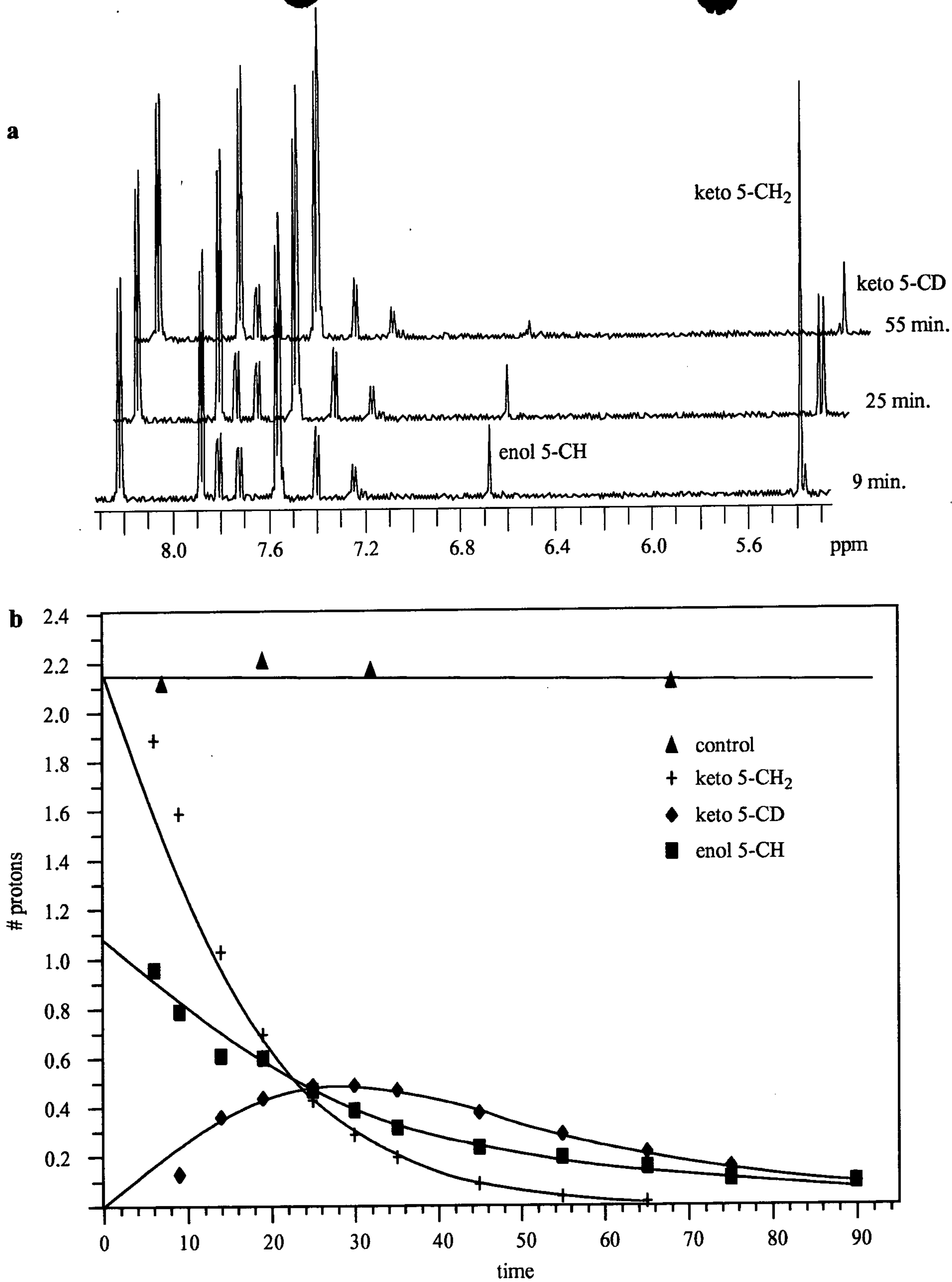
a. The [1]H-DEPT-HMQC spectrum of the mixture from A8(b); CH_2 groups are up, CH and CH_3 down;
b. spectrum of A8(b) shown for reference.



a. The ^1H NMR spectrum of 3-(4-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone in d_6-DMSO/H_2O (89.5/10.5 v/v%);
b. after the addition of DBU (~0.7 equiv.) to (a). The inset is an expansion of the multiplet at 7.32 ppm.



Deuterium exchange of 4-(4-methylsulfonyl)phenyl-3-phenyl-2H-furanone in d_4-MeOH/D_2O (67/33 v/v%) and 33 mM sodium phosphate, pH 7.5; (**a**) observed changes in signal intensity for the 5-CH_2 (5.40 ppm) and 5-CHD (5.38 ppm) protons of the keto form; (**b**) time course of (a).



Deuterium exchange of 3-(4-nitrophenyl)-4-(4-methylsulfonyl)phenyl-2H-furanone in d_6-DMSO/D_2O (80/20 v/v%) and 20 mM sodium phosphate, pH 7.5; (**a**) observed changes in signal intensity for the keto 5-CH_2 (5.40 ppm) , keto 5-CHD (5.38 ppm) and enol 5-CH (6.68 ppm) protons; (**b**) time course of (a).